“ *In vitro* propagation and Conservation of Some Medicinal plants from Lonar forest.”

SYNOPSIS

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YEAR MAY-2016

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1. Introduction:

India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society whether directly as folk medicines or indirectly as pharmaceutical preparation of modern medicine. The use of different parts of several medicinal parts to cure specific diseases has been in vogue from ancient times. The medicinal usage of plants is reported in Indian pharmaceutical codex, Chinese, British and the American pharmacopoeias and in different traditional system of medicines such as Ayurveda, Unani and Siddha.

Many studies have highlighted several pharmacological properties in medicinal plants or their isolated constituents including anti-oxidant, anti-diabetes, antibacterial, antiviral and anti-ulcer activities. Plants have formed the basis of traditional medicine systems that have been in existence for thousands of years. Even in modern times, plant-based systems continue to play an essential role in health care. It has been estimated by the WHO that approximately 80% of the world's population from developing countries rely mainly on traditional medicines for their primary health care. Plant products also play an important role in the health care for the remaining 20% in developing countries, and for those in industrialized countries as well.

Medicinal plants (fruits, vegetables, medicinal herbs, etc.) are a source for a wide variety of natural products, such as the phenolic acids and flavonoids which are very interesting for their antioxidant properties. In addition to their ability to act as an efficient free radical scavengers5 their natural origin represents an advantage to consumer in contrast to synthetic antioxidants which their use is being restricted due to their carcinogenicity6. Epidemiological studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities to a greater or

lesser extent. The intake of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and diseases associated with ageing.

The alkaline Lonar Lake is situated in the village at Lonar, Buldhana district, Maharashtra, India. It ranks third in the world based on diameter and is highly alkaline (pH 10.5). It is a unique saline lake in Asia. Based on the geological studies the formation of the lake is postulated as a meteorite crater, about 50 to 60 thousand year ago. The LonarLake is unique in the world being the only crater formed in basaltic rock on the earth. The lake has circular periphery and is situated in the in a hollow, 0.14km below the ground level with amphitheatre of practically vertical cliffs. In natural alkaline environment, it is observed that LonarLake contains high amount of sodium carbonate, which is a major cause of alkalinity, and in fact, it was used as a source of washing soda in the past. LonarLake is closed system without outlets and regular influents are responsible for its existence.

Based on geological studies, it is postulated that the Lake originated as a meteorite impact crater around 50-60thousand years ago. The diameter of the Lake around the top of the bottom is 1.2Km. Water enters the Lake through rain, ground water seepage and the springs situated in the cliffs at the edge of the Lake. It does not receive any industrial discharges. Alkalinity of the Lake is attributed to the high content of sodium carbonate and hence was used previously as a source of washing soda (Thakker and Ranade, 2002)[1].

Lonar crater is surrounded by dense forests. Many trees are found, such as Custard Apple, Eucalyptus, Lemon grass, Bamboo, Teak, and many trees were seen. It is a place with Rich Bio-Diversity (Malu et al, 2000)[2]. The medicinal value of various fruits and vegetables is known but however, their antibacterial potential is poorly studied (Tambekar and Khadse, 2002)[3].

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1 Thakker, CD. &Ranade, DR. (2002). AlkalophilicMethanosarcina Isolated from Lonar Lake, *Curr. Sci.,* 82:455-458

2 Malu. RA., Dhabhade, DS. &Kodarkar, MS., (2000).*Diversity of Lonar Lake.* J. Aquat Bio., 15:16-18.

3 Tambekar, DH.,&Khadse, V. (2002). Evaluations of antibacterial activity of various plants extract on Salmonella typhi. *Amravati University Research Bulletin.,* 1 (1): 63-65

Medicinal plants and traditional medicine play an important role in the health care system of most developing countries. The traditional health care practice is mainly dependent on medicinal plants collected from the wild. In spite of this, the medicinal plant biodiversity is being depleted due to man-made and natural calamities. Moreover, the indigenous knowledge associated with the conservation and use of medicinal plants is also disappearing at an alarming rate. The fact that medicinal plants could be used as sources of revenue for farmers, the Institute of Biodiversity Conservation (IBC) has initiated the development of a project on Conservation and Sustainable Use of Medicinal Plants (CSMPP). (Singh BM, 2001)[4]

2. Review of Literature

Methabe et al.,(2006) ,reported that,methanol,ethanol,acerone andhot water extract from different part of plants(leaves,eoot ,bark ,stem)of *Indigofera daleoides,punica granatum* and *spirostchys shoerd trmarkable activity* against vibro cholera. **Senthikumar and Reetha, (2009),** trported that methanol extract of Aegle marmelos Cassia species. Showed higher antibacterial activity to a group of bacteria,pathogens.

Bertoni et al., (2013)[5] observed that roots of *Zeyheriamontana*, a species native to the savanna (Cerrado) region of central Brazil, produce lapachol, a naphthoquinone with anticancer activity. Lapachol is also the precursor of β-lapachone, a novel drug candidate for pre- ventive and adjuvant cancer therapies. The leaves of Z. montana are a renewable source of ursolic acid and oleanoic acid, compounds known for their anticancer, antioxidant and antimicrobial properties. The potential prophylactic use of β-lapachone, as well as the medicinal properties of ursolic acid, highlights the importance of this study on Z. montana's germplasm conservation. Multiple shoots were induced on Woody Plant media with supplemented 0.1 mg·L−1 of thidi-azuron (TDZ). Rooting was promoted on half strength WP (Woody Plant) media containing 1.0 mg·L−1 of indole-3- butyric acid (IBA). Plantlet acclimatization to ex-vitro condition was done at a 70% success rate using different sub-strates. It was possible to store Z. montana's elite germplasm using in vitro cultures of media containing 2% sucrose plus 4% sorbitol for six months without subcultures.

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3. Objectives of Research/proposed hypothesis

1) To survey of the medicinal plants from the forest of Lonar Lake.
2) To study the medicinal values of selected medicinal plants from Lonar Lake forest.
3) To propagate the plants in vitro condition.
4) To planting *in-vitroand in-vivo* condition.

4. Methodology

i) Survey:

A survey will be carried out by researcher to identify the medicinal plants available in the forest nearby Lonar Lake.

ii) To Find out Medicinal Value of Plant

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6 Thakker, CD. &Ranade, DR. (2002). AlkalophilicMethanosarcina Isolated from Lonar Lake, *Curr. Sci.,* 82:455-458

7 Malu. RA., Dhabhade, DS. &Kodarkar, MS., (2000).*Diversity of Lonar Lake.* J. Aquat Bio., 15:16-18.

8 Tambekar, DH.,&Khadse, V. (2002). Evaluations of antibacterial activity of various plants extract on Salmonella typhi. *Amravati University Research Bulletin.,* 1 (1): 63-65

The survey will be followed by finding out the medicinal values of identified medicinal plants

iii) Selection of Medicinal Plants for Propagation

Medicinally important and endangered plants will be selected for propagation and conservation out of total identified medicinal plants

iv) Propagation

In vitro propagation has a great potential for germplasm conservation of plant genetic resources. Ex situ conservation using in vitro methods provides a safe repository for plant populations derived from severely at risk locations. Plant parts will be selected for propagation and the propagation will be carried out by following somatic embryogenesis method and Cell Suspension Culture method (Vinoth and Ravindran, 2013)[9]

v) Conservation- 1) *In –situ*- in side natural habitat

2) Ex-siru- outside natural habitat.

5. Importance of Study/Society application

The gradual decline in the population of medicinal plants demand launching of conservation effort so as to ensure continuous and ample supply by establishing a balanced cycle of harvest and renewal. Such conservation efforts would ensure continuous and ample supply of this valuable material which is in great demand by the pharmaceutical industry. Current demand for the medicinal plants has motivated researcher to initiate micro-propagation and in vitro germ-plasm conservation and multiplication. Many medicinal plants are an endangered plant and hence there is a need for its cultivation and conservation.

International status

[9] Vinoth, A. and Ravindhran, R. (2013). In vitro propagation – a potential method for plant Conservation, International Journal of Computing Algorithm, 2: 268-272

Bertoni et al., (2013)[10] observed that roots of *Zeyheriamontana*, a species native to the savanna (Cerrado) region of central Brazil, produce lapachol, a naphthoquinone with anticancer activity. Lapachol is also the precursor of β-lapachone, a novel drug candidate for pre- ventive and adjuvant cancer therapies. The leaves of Z. montana are a renewable source of ursolic acid and oleanoic acid, compounds known for their anticancer, antioxidant and antimicrobial properties. The potential prophylactic use of β-lapachone, as well as the medicinal properties of ursolic acid, highlights the importance of this study on Z. montana's germplasm conservation. Multiple shoots were induced on Woody Plant media with supplemented 0.1 mg·L−1 of thidi-azuron (TDZ). Rooting was promoted on half strength WP (Woody Plant) media containing 1.0 mg·L−1 of indole-3- butyric acid (IBA). Plantlet acclimatization to ex-vitro condition was done at a 70% success rate using different sub-strates. It was possible to store Z. montana's elite germplasm using in vitro cultures of media containing 2% sucrose plus 4% sorbitol for six months without subcultures.

Sadia Bibi et al., (2014)[11] identified, catalogued and documented the large number of medicinal plants used in traditional medicine in Soan Valley, Salt Range, Pakistan.Informal interviews were conducted involving a total of 255 villagers (155 male and 65 female and 35 herbalists) to elicit the knowledge and use of medicinal plants.Local communities possessed knowledge of fifty eight (58) medicinal plant species belonging to thirty five (35) families to treat fifteen ailment categories. Whole plant and leaves were the most frequently used plant parts (24%) followed by seed (14%), root (12%), flower (7%), bulb (6%), fruit (4%), stem (3%), latex and rhizome (2%) and sap and gum (1%). Frequently used growth forms of medicinal plants were wild herbs (63%) followed by cultivated herbs (14%), wild trees (11%), wild shrubs (10%) and wild and cultivated herbs (2%). Preparations were administrated generally through oral and topical routes. Local people were familiar mostly with the species in order to deal common ailments particularly cough, cold, digestive problems, fever,

[10] Bertoni, BW.,Moraes, RM., Previdelli, LL., Pereira, PS., de Castro, F. and Pereira, AMS. (2013). In Vitro Propagation and Conservation of Zeyheriamontana Mart: An Endangered Medicinal Plant, American Journal of Plant Sciences, 4:519-523

[11] Bibi, S., Jawairia, S., Humaria, S. and Malik, RN. (2014) Ethnobotanical uses of medicinal plants in the highlands of Soan Valley, Salt Range, Pakistan, Journal of Ethnopharmacology, 155(1): 352-361

headache, and skin infections. Complex ailments were treated by traditional healers. *Justicaadhatoda, Oleaferruginea, Amaranthusviridis* and *Mentharoyleana* we re identified as plants with high use value (UV).This study revealed that the area harbors high diversity of medicinal flora. Despite gradual socio-cultural transformation, local communities still hold ample knowledge of plants and their uses. The reliance on traditional medicines was associated with the lack of modern health care facilities, poverty and the traditional belief of their effectiveness. Medicinal plants play a significant role in management of various human diseases in the study area. A high degree of consensus among the informants was an indicative that plant use and knowledge were still strong, and preservation of this knowledge showed good foresight in the future. Awareness was thus needed to be raised among local people on sustainable utilization and management of plant resources.

Kose et al., (2015)[12] investigated common ailments in Lesotho's traditional medicine and document plants that are used in treating such ailments. Interviews were conducted in five urban and four rural areas of the capital town of Maseru, by means of questionnaires to elicit information on medicinal plant use to cure common ailments. The informants were 20 males and seven females comprising 15 traditional healers, 11 herbalists and one pharmacist.Reproductive ailments were found to be the most commonly treated, followed by respiratory, degenerative and digestive problems. A list of the 80 plants used for treating the common ailments is given. A total of 44 families is represented, with Asteraceae, Fabaceae, Asphodelaceae and Poaceae families having the highest number of species used for medicinal purposes. The most frequently mentioned medicinal plants in interviews include; Elephantorrhizaelephantina, Pentanisiaprunelloides, Hypoxishermerocallidea,Eriocephalussp.,Salviaruncinata, Scabiosa columbaria, Dicomaanomala, Morella serrata,Xysmalobiumundulatum, and Leobordealanceolata. Due to the high demand of medicinal plants, some species such as L. lanceolata, Tephrosiacapensis, E. elephantina, D. anomala and P. prunelloides were reported as over-harvested. In some

[12] Kose, LS.,Moteetee, A. and Vuuren, S. (2015). Ethnobotanical survey of medicinal plants used in the Maseru district of Lesotho, Journal of Ethnopharmacology, 170: 184-200

cases animal products are added to the medicinal plants to enhance their curative abilities.A total of 80 plants were recorded in the study as treating 38 common ailments in the Maseru district of Lesotho. Records of eight medicinal plants and 146 new medicinal uses of 34 plants that were not recorded elsewhere in literature are reported in the current study for the first time. The new records of medicinal plants used in traditional healing practices in Lesotho clearly show the need to document these practices, and the wealth of new knowledge gained with the current study reinforces the importance of extending the study to other parts of Lesotho.

National Status

Well-developed techniques are currently available to help growers meet the demand of the pharmaceutical industry in the next century. These protocols are designed to provide optimal levels of carbohydrates, organic compounds (vitamins), mineral nutrients, environmental factors (e.g. light, gaseous environment, temperature, and humidity) and growth regulators required to obtain high regeneration rates of many plant species in vitro and thereby facilitate commercially viable micropropagation. Well-defined cell culture methods have also been developed for the production of several important secondary products. Rout et al., (2000)[13] presented an overview of the regeneration of medicinal plants by direct and indirect organogenesis and by somatic embryogenesis from various types of explants is presented, and the use of these techniques combined with other biotechnological approaches to improve medicinal plants through somaclonal variation and genetic transformation is reviewed.

Inularacemosa is an endangered medicinal plant. It is commonly known as Pushkarmool, Pushkar and Manu. The great sage Charaka has characterized it as HikkaMagrahana (stops hiccups) and Savasahara (helpful in asthma). Also, he has cited it as the best medicament for pleurisy along with cough and asthma (http://enwikipedia.org/wiki/charaka-samhita). Due to the fragile nature of its habitat and exploitation due to its commercial medicinal properties, the species are facing the

[13] Rout, GR., Samantaray, S. and Das, P. (2000) In vitro manipulation and propagation of medicinal plants, Biotechnology Advances, 18(2):91-120

onslaught of indiscriminate over-exploitation. So far, this plant has not got the required attention from researchers, hence, except for a few efforts, not much work has been done for its cultivation and conservation. Plant tissue culture offers an attractive and quick method for its multiplication and further conservation. In the present investigation, **Kaur et al., (2010)**[14] developed effective procedures for micro-propagation and in vitro conservation by vitrification. In vitro propagation using aseptically grown seedlings and in vitro conservation via vitrification were standardized. The in vitro conserved material could be retrieved and multiplied normally on MS (Murashige and Skoog, 1962) medium fortified with 1.00 mg l-1BA (benzyl adenine) which has been recorded as the best performing medium for in vitro shoot multiplication. The conserved shoots showed normal in vitro propagation and after retrieval from vitrification platelets were hardened and successfully established in the experimental fields under Nauni (Solan, HP) conditions at an elevation of around 1275 metres above mean sea level.

Conservation of threatened species of medicinal plants and their habitats and Support for livelihood security through protection of wild medicinal plants based on sustainable harvesting. This deals about the promotion of sustainable medicinal plant cultivation through the process of building IPR and field gene bank. Kasagana and **Karumuri (2011)**[15] carried out in-situ conservation of medicinal plants in and around the mountains and national park areas and ex-situ techniques involving cryopreservation and conducting ethno medical survey to explore utilization of medicinal plants. This involves the research on the propagation and cultivation methods of selected indigenous medicinal plants for human and livestock disease. Impact on wild populations of medicinal plants through harvesting and other activities that involves the conservation of medicinal plants on-farm pilot propagation and cultivation trials of medicinal plants on past, present and future scenario. Development and implementation of appropriate management options andguidelines

[14] Kaur, R., Kashyap, A., Majeed, S., Chauhan, N.S., and Bhardwaj, S.B. (2010). In Vitro Propagation and Conservation of InulaRacemosa Hook. F. an Endangered Medicinal Plant of Temperate Origin, Journal of Advanced Laboratory Research in Biology, 1(1): 67-70

[15] Kasagana, VN. andKarumuri, SS. (2011). Conservation Of Medicinal Plants (Past, Present & Future Trends), J. Pharm. Sci. & Res. 3(8):1378-1386

for sustainable harvesting of medicinal plants by applying various conservation techniques. The output will be fieldgene bank established serving research and conservation. Guidelines for sustainable harvest of medicinal plants and itsCultivation practices can be developed by providing income generating activities such as incentives and also creating marketopportunities for both import and export and for formalizing traditional medicine.

In order to meet the increased demand of *Stevia rebaudiana***Thiyagarajan and Venkatachalam (2012)**[16]established an efficient *in vitro* propagation of *S. rebaudiana*. Nodal explants collected from the field were cultured on MS basal medium fortified with different concentrations of BAP (0.5–3.0 mg/l) and KIN (0.5–3.0 mg/l) individually for shoot bud induction. *In vitro* derived nodal buds were cultured on MS medium supplemented with different concentrations (0.5–3.0 mg/l) of BAP and KIN for multiple shoot bud regeneration. In the second experiment, *in vitro* derived buds were placed on MS medium supplemented with different concentrations of BAP (0.5–3.0 mg/l) in combination with 0.5 mg/l IAA or IBA or NAA for shoot bud multiplication. The highest frequency (94.50%) of multiple shoot regeneration with maximum number of shoots (15.69 shoots/explant) was noticed on MS medium supplemented with 1.0 mg/l BAP. For large scale plant production, *in vitro* derived nodal bud explants were cultured on MS medium fortified with 1.0 mg/l BAP, in which about 123 shoots/explant were obtained after three subcultures on the same media composition. Elongated shoots (>2 cm) dissected out from the *in vitro* proliferated shoot clumps were cultured on half-strength MS medium containing different concentrations of NAA (0.1–0.5 mg/l) and/or MS medium fortified with various concentrations (0.5–2.0 mg/l) of auxins (NAA, IAA and IBA) for root induction. Highest frequency of rooting (96%) was noticed on half-strength MS medium augmented with 0.4 mg/l NAA. The rooted plantlets were successfully transferred into plastic cups containing sand and soil in the ratio of 1:2 and subsequently established in the greenhouse. The present *in vitro* propagation protocol

[16] Thiyagarajan, M. and Venkatachalam, P. (2012) Large scale *in vitro* propagation of *Stevia rebaudiana* (bert) for commercial application: Pharmaceutically important and antidiabetic medicinal herb, Industrial Crops and Products, 37(1):111-117

would facilitate an alternative method for rapid and large-scale production of this important antidiabetic medicinal plant.

Pandey et al., (2013)[17] established the protocol for plant regeneration of Psoraleacorylifolia via In-vitro micro propagation. Methods: The Apical meristems was used as the explants cultured on Murashige and Skoog (1962) medium (MS) supplemented different concentrations and combinations of plant growth regulators, 6-Benzylaminopurine (BAP), Kinetin (Kn), 1-Naphthaleneacetic acid (NAA) and B5 vitamins + 2 mg/ltr. Glycine (MBG). Results: Highest Shoot regeneration (95%) results were obtained on MS medium containing BAP (12 μM) with NAA (10.0 μM) and Kn (15.0 μM) generating shoots (6.12 shoots). BAP 12 μM found to be best for shoot multiplication. All the micro-shoots produced normal roots within two weeks of culture on the basic MS medium supplemented with auxin, viz. IAA, IBA or NAA. Maximum of 95% shoots were rooted with an average of 6.8 roots per shoot and average length of 7.11cm on MS medium supplemented with 2.5 μM IBA. Plantlets were transferred to pots where they grew well, attained maturity and set viable seeds. It was concluded that the micro propagation protocol reported here was characterized with a rapid proliferation of shoots, easy rooting of the micro-shoots and the plantlets were easily acclimatized to the external environment and undergoing normal physiological development.

Shekawat et al., (2015)[18] developed an efficient micropropagation protocol successfully for *Morindacoreia* Buch.–Ham. by culturing nodal segments. The explants were washed, sterilized with $HgCl_2$ and inoculated on semi-solid Murashige and Skoog (MS) medium containing various concentrations and combinations of plant growth regulators (PGRs). Shoot bud initiation was observed after one week and 8.6 ± 0.32 shoots (per explant) harvested after five weeks on MS medium with 4.0 mg l^{-1} concentration of 6-benzylaminopurine (BAP). The regenerated shoots were further multiplied on semi-solid MS medium augmented with

[17] Pandey, P. , Mehta, R. and Upadhyay, R. (2013). *In-Vitro* Propagation Of An Endangered Medicinal Plant *PsoraleaCorylifolia*Linn, Asian Journal of Pharmaceutical and Clinical Research, 6(3): 115-118

[18] Shekhawat, MS., Kannan, N. and Manokari, M. (2015) In vitro propagation of traditional medicinal and dye yielding plant *Morindacoreia* Buch.–Ham, South African Journal of Botany, 100: 43-50

2.0 mg l^{-1} BAP + 1.0 mg l^{-1} Kinetin (Kin). Maximum 24.5 ± 0.34 shoots per explant was obtained after five weeks on this media combination. The long (4–5 cm) and healthy shoots were rooted in vitro with 100% success rate on half strength MS medium + 1.0 mg l^{-1} indole-3 butyric acid (IBA). Rooting and acclimatization were achieved simultaneously by ex vitro rooting method using 200 mg l^{-1} IBA for 5 min with very good success rate (28.67 ± 05.51 roots per shoot with 100% response). The rooted shoots were transferred to the greenhouse for acclimatization for 4–5 weeks. The hardened plantlets were finally shifted to the field for further growth in the natural conditions after another five weeks. This is the first report on micropropagation of *M. coreia*, which can be successfully used for the large-scale multiplication and conservation of germplasm of this important medicinal plant.

It was evident from the review of literature that various research effort were made in the field of in vitro propagation and conservation of medicinal plants in India and abroad. Still there is no effective research carried out on the similar topics in Vidarbha region particularly for the medicinal plants observed in the forest near by Lonar Lake. Hence researcher decided to carry out research on the above topic.

6. Proposed Work Plan of Study:

Ist Year-

1) Review of Literature
2) Selection of Explant
3) Collection of Explant Samples

IInd Year-

1) Surface disinfection of explant material
2) Media composition and preparation
3) Transfer of Explant
4) Growing Explant

III rd Year

1) Conservation- 1) In-situ-

2) Ex-situ

2) Transplanting

3) Data analysis

4) Submission of thesis.

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